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1. Document ID: US 20040072158 A1

L6: Entry 1 of 10

File: PGPB

Apr 15, 2004

DOCUMENT-IDENTIFIER: US 20040072158 A1

TITLE: Electrochemical detection of nucleic acid sequences

Detail Description Paragraph:

[0223] Techniques for allele-specific amplification of Factor V wild-type and mutant genes have been described (Kirschbaum and Foster, 1995). The allele-specific amplification of Factor V genes has been repeated followed with electrochemical detection using Rapid PCR.TM. detection methodology. The forward primer has the same sequence as that reported by Kirschbaum and Foster, except that it is labeled with a biotin at the 5'-end. Two discriminating reverse primers have the same sequences as those reported by Kirschbaum and Foster, but are modified with a 5'-fluorescein. These primers are discriminatory based on homology of the Y-nucleotide of the reverse primers with either the wild-type or the mutant DNA sequence. PCR amplification was performed as described by Kirschbaum and Foster (1995), and amplification products were captured directly onto sensors through the biotin:avidin interaction. The double-stranded DNA was conjugated with anti-fluorescein HRP, washed and treated with the substrate and mediator to generate a current. PCR amplification products were also monitored by agarose gel electrophoresis to confirm that double-stranded products generated are of the predicted size. Null DNA controls and controls of known genotype were included as well. A high signal is expected only when the discriminating primer finds homology with one of the alleles in the sample.

Detail Description Paragraph:

[0465] Techniques for allele-specific amplification of Factor V wild-type and mutant genes have been described (Kirschbaum and Foster, 1995). Therefore, initial experiments were aimed at repeating, the allele-specific amplification of Factor V genes, followed with electrochemical detection using our direct detection methodology. The forward primer has the same sequence as that reported by Kirschbaum and Foster, except that it is labeled with a biotin at the 5'-end. Two discriminating reverse primers have the same sequences as those reported by Kirschbaum and Foster, but are modified with a 5'-fluorescein. These primers are discriminatory based on identity of the 3'-nucleotide with either the wild-type or the mutant Factor V gene sequence. PCR amplification was performed as described by Kirschbaum and Foster, and amplification products were captured directly onto sensors through the biotin:avidin interaction as described. The double-stranded DNA was conjugated with anti-fluorescein HRP, washed and treated with the substrate and mediator to generate a current. PCR amplification products will also be monitored by agarose gel electrophoresis to confirm that double-stranded products generated are of the predicted size. Null DNA controls and controls of known genotype will be included as well. A high signal from normal individuals is expected with only a background signal from homozygous carriers. After conditions for amplification and

assay have been determined for Factor V, various types of samples will be tested. Samples from individuals known to be normal for the Factor V gene mutation, heterozygous for the mutation and homozygous for the mutation (provided by L. Silverman) were analyzed. In a blind study the genotype at the factor V locus was correctly determined in 19 samples.

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KWIC	Drawn D
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2. Document ID: US 20040038194 A1

L6: Entry 2 of 10

File: PGPB

Feb 26, 2004

DOCUMENT-IDENTIFIER: US 20040038194 A1

TITLE: Diagnostic polymerase chain reaction process utilizing simultaneous capture and detection of amplicons

Abstract Paragraph:

A method simultaneously detects and captures double-stranded DNA sequence. The method includes providing a sample. The next step is adding a forward primer for the double-stranded DNA sequence and a reverse primer for the double-stranded DNA sequence; either the forward primer or the reverse primer have a capture agent, the other has a detection agent. The next step is replicating the double-stranded DNA sequence. The next step is binding the capture agent to a capture medium. The next step is rinsing the sample. The next step is detecting the detection agent. The method also can be applied to double-stranded DNA that has been reverse-transcribed from single-stranded RNA.

Summary of Invention Paragraph:

[0005] It is an object of the invention to provide a diagnostic polymerase chain reaction utilizing simultaneous capture and detection of amplicons that overcomes the disadvantages of the heretofore-known devices and methods of this general type. With the foregoing and other objects in view there is provided, in accordance with the invention, a method for simultaneously detecting and capturing a double-stranded DNA sequence. The method includes the following steps. The first step is providing a sample. The next step is adding a forward primer for the double-stranded DNA sequence and a reverse primer for the double-stranded DNA sequence. Either the forward primer or the reverse primer have a capture agent; the other has a detection agent. The next step is replicating the double-stranded DNA sequence. The next step is binding the capture agent to a capture medium. The next step is rinsing the sample. The next step is detecting the detection agent.

Summary of Invention Paragraph:

[0012] In accordance with a further object of the invention, the method includes detecting a plurality of double-stranded DNA sequence by adding a forward primer and a reverse primer for each additional double-stranded DNA sequence. One of each pair of a forward primers and a reverse primer has a capture agent, and the other of the pair has a second detection agent.

Summary of Invention Paragraph:

[0020] In accordance with a further object of the invention, the method includes binding the capture agent to a mobile phase. With the objects of the invention in view, there is also provided a method for simultaneously detecting and capturing a double-stranded DNA sequence complementing a single-stranded RNA sequence. The method includes the following steps. The first step is providing a single-stranded RNA sequence. The next step is adding a forward primer complementing the

single-stranded RNA. The next step is reverse transcribing the single-stranded RNA to produce a double-stranded DNA sequence. The next step is adding a reverse primer for the double-stranded DNA sequence; the forward primer or the reverse primer have a capture agent and the other has a detection agent. The next step is replicating the double-stranded DNA sequence. The next step is binding the capture agent to a capture medium. The next step is rinsing the sample. The next step is detecting the detection agent.

CLAIMS:

1. A method for simultaneously detecting and capturing a double-stranded DNA sequence, which comprises: providing a sample; adding a forward primer for the double-stranded DNA sequence and a reverse primer for the double-stranded DNA sequence; one of the forward primer and the reverse primer having a capture agent, the other of the forward primer and the reverse primer having a detection agent; replicating the double-stranded DNA sequence; binding the capture agent to a capture medium; rinsing the sample; and detecting the detection agent.
10. The method according to claim 1, which further comprises: detecting a plurality of double-stranded DNA sequences by: adding a forward primer and a reverse primer for each additional double-stranded DNA sequence; one of each pair of a forward primer and a reverse primer having a capture agent, and the other of the pair having a second detection agent, each detection agent being different.
18. A method for simultaneously detecting and capturing a double-stranded DNA sequence complementing a single-stranded RNA sequence, which comprises: providing a single-stranded RNA sequence; adding a forward primer complementing the single-stranded RNA; reverse transcribing the single-stranded RNA to produce a double-stranded DNA sequence; adding a reverse primer for the double-stranded DNA sequence; one of the forward primer and the reverse primer having a capture agent, the other of the forward primer and the reverse primer having a detection agent; replicating the double-stranded DNA sequence; binding the capture agent to a capture medium; rinsing the sample; and detecting the detection agent.

[Full](#) | [Title](#) | [Citation](#) | [Front](#) | [Review](#) | [Classification](#) | [Date](#) | [Reference](#) | [Sequences](#) | [Attachments](#) | [Claims](#) | [KIMC](#) | [Drawn D](#)

3. Document ID: US 20030224418 A1

L6: Entry 3 of 10

File: PGPB

Dec 4, 2003

DOCUMENT-IDENTIFIER: US 20030224418 A1

TITLE: Genes and polymorphisms associated with cardiovascular disease and their use

Detail Description Paragraph:

[0206] PCR primers were synthesized by Operon (Alameda, Calif.) using phosphoramidite chemistry. Amplification of the COX6B target sequence was carried out in two 50 .mu.l PCR reactions with 100 ng of pooled human genomic DNA, obtained as described in Example 1, taken from samples in pool 1 or pool 2, although amounts ranging from 100 ng to 1 ug could be used. Individual DNA concentrations within the pooled samples were present in equal concentration with a final concentration of 0.5 ng. Each reaction contained 1.times.PCR buffer (Qiagen, Valencia, Calif.), 200 .mu.M dNTPs, 1U Hotstar Taq polymerase (Qiagen, Valencia, Calif.), 4 mM MgCl<sub>2</sub>, and 25 pmols of the long primer containing both the universal primer sequence and the target specific sequence 5'-AGCCGATAACAATTTCACACA-GGTAGTCTGGTTCTGGTTGGGG-3' (SEQ ID NO.: 4), 2 pmoles of the short primer 5'-

AGGATTTCAGCACCATGGC-3' (SEQ ID NO.: 3) and 10 pmoles of a biotinylated universal primer complementary to the 5' end of the PCR amplicon 5'-AGCGGATAACAATTTCACACAGG-3' (SEQ ID NO.: 121). Alternatively, the biotinylated universal primer could be 5'-GGCGCACGCCTCCACG-3' (SEQ ID NO.: 122). After an initial round of amplification with the target with the specific forward (long) and reverse primer (short), the 5' biotinylated universal primer then hybridized and acted as a reverse primer thereby introducing a 3' biotin capture moiety into the molecule. The amplification protocol results in a 5'-biotinylated double stranded DNA amplicon and dramatically reduces the cost of high throughput genotyping by eliminating the need to 5' biotin label each forward primer used in a genotyping. Thermal cycling was performed in 0.2 mL tubes or 96 well plate using an MJ Research Thermal Cycler (Waltham, Mass.) (calculated temperature) with the following cycling parameters: 94.degree. C. for 5 min; 45 cycles: 94.degree. C. for 20 sec, 56.degree. C. for 30 sec, 72.degree. C. for 60 sec; 72.degree. C. 3 min.

Detail Description Paragraph:

[0216] PCR primers were synthesized by Operon (Alameda, Calif.) using phosphoramidite chemistry. Amplification of the GPI-1 target sequence was carried out in single 50 .mu.l PCR reaction with 100 ng of pooled human genomic DNA (200 samples), obtained as described in Example 1, taken from samples in pool 3 or pool 4, although amounts ranging from 100 ng to 1 ug could be used. Individual DNA concentrations within the pooled samples were present in equal concentration with the final concentration of 0.5 ng. Each reaction contained 1.times.PCR buffer (Qiagen, Valencia, Calif.), 200 uM dNTPs, 1U Hotstar Taq polymerase (Qiagen, Valencia, Calif.), 4 mM MgCl<sub>2</sub>, and 25 pmols of the forward primer containing both the universal primer sequence and the target specific short sequence 5'-AGCAGGGCTTCCTCCTTC-3' (SEQ ID NO.: 8) 2 pmoles of the long primer 5'-AGCGGATAACAATTTCACACAGGTGACCCAGCCGTACCTATTC-3' (SEQ ID NO.: 9) and 10 pmoles of a biotinylated universal primer complementary to the 5' end of the PCR amplicon 5'-AGCGGATAACAATTTCACACAGG-3' (SEQ ID NO.: 121). After an initial round of amplification with the target with the specific forward (long) and reverse primer (short), the 5' biotinylated universal primer then hybridized and acted as a reverse primer thereby introducing a 3' biotin capture moiety into the molecule. The amplification protocol results in a 5'-biotinylated double stranded DNA amplicon and dramatically reduces the cost of high throughput genotyping by eliminating the need to 5' biotin label each forward primer used in a genotyping. Thermal cycling was performed in 0.2 mL tubes or 96 well plate using an MJ Research Thermal Cycler (Waltham, Mass.) (calculated temperature) with the following cycling parameters: 94.degree. C. for 5 min; 45 cycles: 94.degree. C. for 20 sec, 56.degree. C. for 30 sec, 72.degree. C. for 60 sec; 72.degree. C. 3 min.

Full		Title		Citation		Front		Review		Classification		Date		Reference		Sequences		Attachments		Claims		KWIC		Drawn Ds
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4. Document ID: US 20030207297 A1

L6: Entry 4 of 10

File: PGPB

Nov 6, 2003

DOCUMENT-IDENTIFIER: US 20030207297 A1

TITLE: Methods for generating databases and databases for identifying polymorphic genetic markers

Detail Description Paragraph:

[0344] 5'-AGCGGATAACAATTTCACACAGG-3' (SEQ ID NO: 47). After an initial round of amplification with the target with the specific forward and reverse primer, the 5' biotinylated universal primer then hybridized and acted as a reverse primer thereby

introducing a 3' biotin capture moiety into the molecule. The amplification protocol results in a 5'-biotinylated double stranded DNA amplicon and dramatically reduces the cost of high throughput genotyping by eliminating the need to 5' biotin label each forward primer used in a genotyping. Thermal cycling was performed in 0.2 mL tubes or 96 well plate using an MJ Research Thermal Cycler (calculated temperature) with the following cycling parameters: 94.degree. C. for 5 min; 45 cycles: 94.degree. C. for 20 sec, 56.degree. C. for 30 sec, 72.degree. C. for 60 sec; 72.degree. C. 3 min.

Detail Description Paragraph:

[0362] After an initial round of amplification with the target with the specific forward and reverse primer, the 5' biotinylated universal primer can then be hybridized and acted as a forward primer thereby introducing a 5' biotin capture moiety into the molecule. The amplification protocol resulted in a 5'-biotinylated double stranded DNA amplicon and dramatically reduced the cost of high throughput genotyping by eliminating the need to 5' biotin label every forward primer used in a genotyping.

Detail Description Paragraph:

[0378] 5'-AGCGGATAACAATTTCACACAGG-3' (SEQ ID NO: 51). After an initial round of amplification of the target with the specific forward and reverse primers, the 5' biotinylated universal primer was then hybridized and acted as a reverse primer thereby introducing a 3' biotin capture moiety into the molecule. The amplification protocol results in a 5'-biotinylated double stranded DNA amplicon and dramatically reduces the cost of high throughput genotyping by eliminating the need to 5' biotin label each forward primer used in a genotyping. Thermal cycling was performed in 0.2 mL tubes or 96 well plate using an MJ Research Thermal Cycler (calculated temperature) with the following cycling parameters: 94.degree. C. for 5 min; 45 cycles: 94.degree. C. for 20 sec, 56.degree. C. for 30 sec, 72.degree. C. for 60 sec; 72.degree. C. 3 min.

[Full](#) | [Title](#) | [Citation](#) | [Front](#) | [Review](#) | [Classification](#) | [Date](#) | [Reference](#) | [Sequences](#) | [Attachments](#) | [Claims](#) | [KWIC](#) | [Drawn D](#)

□ 5. Document ID: US 20030190644 A1

L6: Entry 5 of 10

File: PGPB

Oct 9, 2003

DOCUMENT-IDENTIFIER: US 20030190644 A1

TITLE: Methods for generating databases and databases for identifying polymorphic genetic markers

Detail Description Paragraph:

[0339] PCR primers were synthesized by OPERON using phosphoramidite chemistry. Amplification of the AKAP10 target sequence was carried out in single 50 .mu.l PCR reaction with 100 ng-1 ug of pooled human genomic DNAs in a 50 .mu.l PCR reaction. Individual DNA concentrations within the pooled samples were present in equal concentration with the final concentration ranging from 1-25 ng. Each reaction containing IX PCR buffer (Qiagen, Valencia, Calif.), 200 uM dNTPs, 1U Hotstar Taq polymerase (Qiagen, Valencia, Calif.), 4 mM MgCl<sub>2</sub>.sub.2, and 25 pmol of the forward primer containing the universal primer sequence and the target specific sequence 5'-TCTCAATCATGTGCATTGAGG-3' (SEQ ID NO: 45), 2 pmol of the reverse primer 5'-AGCGGATAACAATTTCACACAGGGATCACACAGCCATCAGCAG- -3' (SEQ ID NO: 46), and 10 pmol of a biotinylated universal primer complementary to the 5' end of the PCR amplicon 5'-AGCGGATAACAATTTCACACAG- G-3' (SEQ ID NO: 47). After an initial round of amplification with the target with the specific forward and reverse primer, the 5'

biotinylated universal primer then hybridized and acted as a reverse primer thereby introducing a 3' biotin capture moiety into the molecule. The amplification protocol results in a 5'-biotinylated double stranded DNA amplicon and dramatically reduces the cost of high throughput genotyping by eliminating the need to 5' biotin label each forward primer used in a genotyping. Thermal cycling was performed in 0.2 mL tubes or 96 well plate using an MJ Research Thermal Cycler (calculated temperature) with the following cycling parameters: 94.degree. C. for 5 min; 45 cycles: 94.degree. C. for 20 sec, 56.degree. C. for 30 sec, 72.degree. C. for 60 sec; 72.degree. C. 3 min.

Detail Description Paragraph:

[0355] PCR primers were synthesized by OPERON using phosphoramidite chemistry. Amplification of the AKAP10 target sequence was carried out in a single 50 .mu.l PCR reaction with 100 ng-1 .mu.g of pooled human genomic DNAs in a 50 .mu.l PCR reaction. Individual DNA concentrations within the pooled samples were present in equal concentration with the final concentration ranging from 1-25 ng. Each reaction contained 1.times. PCR buffer (Qiagen, Valencia, Calif.), 200 .mu.M dNTPs, 1U Hotstar Taq polymerase (Qiagen, Valencia, Calif.), 4 mM MgCl<sub>2</sub>, and 25 pmol of the forward primer containing the universal primer sequence and the target specific sequence 5'-AGCGGATAACAATTCACACAGGGAGCTAGCTTGGAA- GAT TGC-3' (SEQ ID NO: 41), 2 pmol of the reverse primer 5'-GTCCAATATATGCAAACAGTTG-3' (SEQ ID NO: 54), and 10 pmol of a biotinylated universal primer complementary to the 5' end of the PCR amplicon BIO:5'-AGCGGATAACAATTCACACAGG-3' (SEQ ID NO: 43). After an initial round of amplification with the target with the specific forward and reverse primer, the 5' biotinylated universal primer can then be hybridized and acted as a forward primer thereby introducing a 5' biotin capture moiety into the molecule. The amplification protocol resulted in a 5'-biotinylated double stranded DNA amplicon and dramatically reduced the cost of high throughput genotyping by eliminating the need to 5' biotin label every forward primer used in a genotyping.

Detail Description Paragraph:

[0368] PCR primers were synthesized by OPERON using phosphoramidite chemistry. Amplification of the AKAP10 target sequence was carried out in single 50 .mu.l PCR reaction with 100 ng-1 ug of pooled human genomic DNA templates in a 50 .mu.l PCR reaction. Individual DNA concentrations within the pooled samples were present in an equal concentration with the final concentration ranging from 1-25 ng. Each reaction containing I X PCR buffer (Qiagen, Valencia, Calif.), 200 .mu.M dNTPs, 1U Hotstar Taq polymerase (Qiagen, Valencia, Calif.), 4 mM MgCl<sub>2</sub>, 25 pmol of the forward primer containing the universal primer sequence and the target specific sequence 5'-TTTCTCTGCACAGAGAGGC-3' (SEQ ID NO: 49), 2 pmol of the reverse primer 5'-AGCGGATAACAATTCACACAGGGCTGAAATCCTCGCTTACC-3' (SEQ ID NO: 50), and 10 pmol of a biotinylated universal primer complementary to the 5' end of the PCR amplicon 5'-AGCGGATAACAATTCACACAG- G-3' (SEQ ID NO: 51). After an initial round of amplification of the target with the specific forward and reverse primers, the 5' biotinylated universal primer was then hybridized and acted as a reverse primer thereby introducing a 3' biotin capture moiety into the molecule. The amplification protocol results in a 5'-biotinylated double stranded DNA amplicon and dramatically reduces the cost of high throughput genotyping by eliminating the need to 5' biotin label each forward primer used in a genotyping. Thermal cycling was performed in 0.2 mL tubes or 96 well plate using an MJ Research Thermal Cycler (calculated temperature) with the following cycling parameters: 94.degree. C. for 5 min; 45 cycles: 94.degree. C. for 20 sec, 56.degree. C. for 30 sec, 72.degree. C. for 60 sec; 72.degree. C. 3 min.

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KWIC	Drawn D
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6. Document ID: US 20030186251 A1

L6: Entry 6 of 10

File: PGPB

Oct 2, 2003

DOCUMENT-IDENTIFIER: US 20030186251 A1

TITLE: Genome sequence tags

Detail Description Paragraph:

[0045] Following centrifugation, the pellet was washed in 70% ethanol, dried and then dissolved in 200  $\mu$ l TE<sub>S1</sub>. A portion ({fraction (1/25)}th) was subjected to 25 additional rounds of linear amplification under the above LARHD conditions, except only the forward primer was added. This was then followed by one round of amplification after addition of the reverse primer and additional DNA polymerase to convert the linear amplification products to double-stranded DNA. Typically, 1 ml of sample is amplified and any unincorporated primers are hydrolyzed by incubation with Exo I as above. After P/C extraction and ethanol precipitation, the amplified DNA is digested with 20 U of NlaIII in 300  $\mu$ l at 37.degree. C. for 4 h (after 2 h the completion of digestion is checked by electrophoresis of a small aliquot on a 10% polyacrylamide gel). The digest is then extracted on ice with chilled P/C to prevent denaturation of the smaller GSTs and ethanol precipitated from Na acetate in the presence of Glyco Blue carrier. The sample is chilled for several h and then centrifuged at 4.degree. C. The pellets are resuspended in 200  $\mu$ l ice cold TE<sub>S1</sub> plus 25 mM NaCl, diluted with an equal volume of 2.times.MBB and added to 200  $\mu$ l (2 mg) of streptavidin beads equilibrated with 1.times.MBB. After gentle mixing for 15 m at room temperature, the unbound fraction is transferred to a second 200  $\mu$ l aliquot of beads to capture any remaining biotinylated DNA fragments. The unbound GST fraction is recovered and precipitated by addition of 2.5 volume of ethanol and Glyco Blue carrier and concatemerized with T4 DNA ligase (5 U./ $\mu$ l, Invitrogen) at 16.degree. C. for 6 hr. The sample was subjected to electrophoresis on a 0.75% low melt agarose gel and products greater than 100 bp were recovered. These products were cloned into the SphI-site of a pZero plasmid (Invitrogen) that was engineered to have a SphI-minus KanR gene (unpublished). Recombinant clones obtained after electroporation of competent TOP10 cells (Invitrogen, Carlsbad, Calif.) are selected on 2.times.YT plates containing 50  $\mu$ g/ml kanamycin. A schematic representation of the method is shown in FIG. 1 and a complete description of all steps is available at the web site (in preparation).

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KWIC	Drawn Ds
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 7. Document ID: US 20030180748 A1

L6: Entry 7 of 10

File: PGPB

Sep 25, 2003

DOCUMENT-IDENTIFIER: US 20030180748 A1

TITLE: Methods for generating databases and databases for identifying polymorphic genetic markers

Detail Description Paragraph:

[0328] PCR primers were synthesized by OPERON using phosphoramidite chemistry. Amplification of the AKAP10 target sequence was carried out in single 50  $\mu$ l PCR reaction with 100 ng-1 ug of pooled human genomic DNAs in a 50  $\mu$ l PCR reaction. Individual DNA concentrations within the pooled samples were present in equal

concentration with the final concentration ranging from 1-25 ng. Each reaction containing 1.times.PCR buffer (Qiagen, Valencia, Calif.), 200 uM dNTPs, 1U Hotstar Taq polymerase (Qiagen, Valencia, Calif.), 4 mM MgCl<sub>2</sub>, and 25 pmol of the forward primer containing the universal primer sequence and the target specific sequence 5'-TCTCAATCATGTGCATTGAGG-3' (SEQ ID NO: 45), 2 pmol of the reverse primer 5'-AGCGGATAACAATTTCACACAGGGATCACACAGCCATCAGCAG- -3' (SEQ ID NO: 46), and 10 pmol of a biotinylated universal primer complementary to the 5' end of the PCR amplicon 5'-AGCGGATAACAATTTCACACAG- G-3' (SEQ ID NO: 47). After an initial round of amplification with the target with the specific forward and reverse primer, the 5' biotinylated universal primer then hybridized and acted as a reverse primer thereby introducing a 3' biotin capture moiety into the molecule. The amplification protocol results in a 5'-biotinylated double stranded DNA amplicon and dramatically reduces the cost of high throughput genotyping by eliminating the need to 5' biotin label each forward primer used in a genotyping. Thermal cycling was performed in 0.2 mL tubes or 96 well plate using an MJ Research Thermal Cycler (calculated temperature) with the following cycling parameters: 94.degree. C. for 5 min; 45 cycles: 94.degree. C. for 20 sec, 56.degree. C. for 30 sec, 72.degree. C. for 60 sec; 72.degree. C. 3 min.

Detail Description Paragraph:

[0344] PCR primers were synthesized by OPERON using phosphoramidite chemistry. Amplification of the AKAP10 target sequence was carried out in a single 50 .mu.l PCR reaction with 100 ng-1 .mu.g of pooled human genomic DNAs in a 50 .mu.l PCR reaction. Individual DNA concentrations within the pooled samples were present in equal concentration with the final concentration ranging from 1-25 ng. Each reaction contained 1.times.PCR buffer (Qiagen, Valencia, Calif.), 200 .mu.M dNTPs, 1U Hotstar Taq polymerase (Qiagen, Valencia, Calif.), 4 mM MgCl<sub>2</sub>, and 25 pmol of the forward primer containing the universal primer sequence and the target specific sequence 5'-AGCGGATAACAATTTCACACAGGGAGCTAGCTTGGAA- GATTGC-3' (SEQ ID NO: 41), 2 pmol of the reverse primer 5'-GTCCAATATATGCAAACAGTTG-3' (SEQ ID NO: 54), and 10 pmol of a biotinylated universal primer complementary to the 5' end of the PCR amplicon BIO:5'-AGCGGATAACAATTTCACACAGG-3' (SEQ ID NO: 43). After an initial round of amplification with the target with the specific forward and reverse primer, the 5' biotinylated universal primer can then be hybridized and acted as a forward primer thereby introducing a 5' biotin capture moiety into the molecule. The amplification protocol resulted in a 5'-biotinylated double stranded DNA amplicon and dramatically reduced the cost of high throughput genotyping by eliminating the need to 5' biotin label every forward primer used in a genotyping.

Detail Description Paragraph:

[0357] PCR primers were synthesized by OPERON using phosphoramidite chemistry. Amplification of the AKAP10 target sequence was carried out in single 50 .mu.l PCR reaction with 100 ng-1 ug of pooled human genomic DNA templates in a 50 .mu.l PCR reaction. Individual DNA concentrations within the pooled samples were present in an equal concentration with the final concentration ranging from 1-25 ng. Each reaction containing 1.times.PCR buffer (Qiagen, Valencia, Calif.), 200 .mu.M dNTPs, 1U Hotstar Taq polymerase (Qiagen, Valencia, Calif.), 4 mM MgCl<sub>2</sub>, 25 pmol of the forward primer containing the universal primer sequence and the target specific sequence 5'-TTCTCTGCACAGAGAGGC-3' (SEQ ID NO: 49), 2 pmol of the reverse primer 5'-AGCGGATAACAATTTCACACAGGGCTGAAATCCTCGCTTAC- C-3' (SEQ ID NO: 50), and 10 pmol of a biotinylated universal primer complementary to the 5' end of the PCR amplicon 5'-AGCGGATAACAATTTCACACAG- G-3' (SEQ ID NO: 51). After an initial round of amplification of the target with the specific forward and reverse primers, the 5' biotinylated universal primer was then hybridized and acted as a reverse primer thereby introducing a 3' biotin capture moiety into the molecule. The amplification protocol results in a 5'-biotinylated double stranded DNA amplicon and dramatically reduces the cost of high throughput genotyping by eliminating the need to 5' biotin label each forward primer used in a genotyping. Thermal cycling was performed in 0.2 mL tubes or 96 well plate using an MJ Research Thermal Cycler (calculated temperature) with the following cycling parameters: 94.degree. C. for 5 min; 45

cycles: 94.degree. C. for 20 sec, 56.degree. C. for 30 sec, 72.degree. C. for 60 sec; 72.degree. C. 3 min.

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KWIC	Draw D
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8. Document ID: US 20030036057 A1

L6: Entry 8 of 10

File: PGPB

Feb 20, 2003

DOCUMENT-IDENTIFIER: US 20030036057 A1

TITLE: Genes and polymorphisms associated with cardiovascular disease and their use

Detail Description Paragraph:

[0206] DNA samples (as prepared in Example 1), representing 200 women, from the lower extreme, pool 1 (low levels of cholesterol) and the upper extreme, pool 2 (high levels of cholesterol) were amplified and analyzed for genetic differences using a MassEXTEND.TM. assay detection method. For each pool, single nucleotide polymorphisms were examined throughout the entire genome to detect differences in allelic frequency of a variant allele between the pools. PCR Amplification of Samples from Pools 1 and 2 PCR primers were synthesized by Operon (Alameda, Calif.) using phosphoramidite chemistry. Amplification of the COX6B target sequence was carried out in two 50 .mu.l PCR reactions with 100 ng of pooled human genomic DNA, obtained as described in Example 1, taken from samples in pool 1 or pool 2, although amounts ranging from 100 ng to 1 ug could be used. Individual DNA concentrations within the pooled samples were present in equal concentration with a final concentration of 0.5 ng. Each reaction contained 1.times. PCR buffer (Qiagen, Valencia, Calif.), 200.mu.M dNTPs, 1 U Hotstar Taq polymerase (Qiagen, Valencia, Calif.), 4 mM MgCl<sub>2</sub>, and 25 pmols of the long primer containing both the universal primer sequence and the target specific sequence 5'-AGCGGATAACAATTTCACACAGGTAGTCTGGTTCTGGTTGGGG-3' (SEQ ID NO.: 4), 2 pmoles of the short primer 5'-AGGATTTCAGCACCAGGC-3' (SEQ ID NO.: 3) and 10 pmol of a biotinylated universal primer complementary to the 5' end of the PCR amplicon 5'-AGCGGATAACAATTTCACACAGG-3' (SEQ ID NO.: 121). Alternatively, the biotinylated universal primer could be 5'-GGCGCACGCCCTCCACG-3' (SEQ ID NO.: 122). After an initial round of amplification with the target with the specific forward (long) and reverse primer (short), the 5' biotinylated universal primer then hybridized and acted as a reverse primer thereby introducing a 3' biotin capture moiety into the molecule. The amplification protocol results in a 5'-biotinylated double stranded DNA amplicon and dramatically reduces the cost of high throughput genotyping by eliminating the need to 5' biotin label each forward primer used in a genotyping. Thermal cycling was performed in 0.2 mL tubes or 96 well plate using an MJ Research Thermal Cycler (Waltham, Mass.) (calculated temperature) with the following cycling parameters: 94.degree. C. for 5 min; 45 cycles: 94.degree. C. for 20 sec, 56.degree. C. for 30 sec, 72.degree. C. for 60 sec; 72.degree. C. 3 min.

Detail Description Paragraph:

[0214] PCR primers were synthesized by Operon (Alameda, Calif.) using phosphoramidite chemistry. Amplification of the GPI-1 target sequence was carried out in single 50 .mu.l PCR reaction with 100 ng of pooled human genomic DNA (200 samples), obtained as described in Example 1, taken from samples in pool 3 or pool 4, although amounts ranging from 100 ng to 1 ug could be used. Individual DNA concentrations within the pooled samples were present in equal concentration with the final concentration of 0.5 ng. Each reaction contained 1.times. PCR buffer (Qiagen, Valencia, Calif.), 200 uM dNTPs, 1 U Hotstar Taq polymerase (Qiagen, Valencia, Calif.), 4 mM MgCl<sub>2</sub>, and 25 pmols of the forward primer containing

both the universal primer sequence and the target specific short sequence 5'-AGCAGGGCTTCCTCTTC-3' (SEQ ID NO.: 8) 2 pmoles of the long primer 5'-AGCGGATAACAAATTTCACACAGGTGACCCAGCCGTACCTATTC-3' (SEQ ID NO.: 9) and 10 pmoles of a biotinylated universal primer complementary to the 5' end of the PCR amplicon 5'-AGCGGATAACAAATTTCACACAGG-3' (SEQ ID NO.: 121). After an initial round of amplification with the target with the specific forward (long) and reverse primer (short), the 5' biotinylated universal primer then hybridized and acted as a reverse primer thereby introducing a 3' biotin capture moiety into the molecule. The amplification protocol results in a 5'-biotinylated double stranded DNA amplicon and dramatically reduces the cost of high throughput genotyping by eliminating the need to 5' biotin label each forward primer used in a genotyping. Thermal cycling was performed in 0.2 mL tubes or 96 well plate using an MJ Research Thermal Cycler (Waltham, Mass.) (calculated temperature) with the following cycling parameters: 94.degree. C. for 5 min; 45 cycles: 94.degree. C. for 20 sec, 56.degree. C. for 30 sec, 72.degree. C. for 60 sec; 72.degree. C. 3 min.

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9. Document ID: US 20020040130 A1

L6: Entry 9 of 10

File: PGPB

Apr 4, 2002

DOCUMENT-IDENTIFIER: US 20020040130 A1

TITLE: Polymorphic kinase anchor proteins and nucleic acids encoding the same

Detail Description Paragraph:

[0387] AKAP10-1 is an allele of the AKAP10 gene with a single nucleotide polymorphism at nucleotide number 156277 (based on the sequence of a genomic clone of the AKAP10 gene, GenBank Accession No. AC005730). The single nucleotide polymorphism is a T to C transversion located in the 3'non-translated region of the gene encoding AKAP10. PCR primers were synthesized by OPERON (Alameda, Calif.) using phosphoramidite chemistry. Amplification of the AKAP10 target sequence was carried out in single 50 .mu.l PCR reaction with 25 ng of human genomic DNA obtained from samples as described in Example 1. Each reaction containing IX PCR buffer (Qiagen, Valencia, Calif.), 200 .mu.M dNTPs, 1U Hotstar Taq polymerase (Qiagen, Valencia, Calif.), 4 mM MgCl<sub>2</sub>, and 25 pmols of the forward primer containing the universal primer sequence and the target specific sequence 5'-TCTCAATCATGTGCATTGAGG-3' (SEQ ID NO: 5) 2 pmoles of the reverse primer 5'-AGCGGATAACAAATTTCACACAGGGATCACACAGCCATCAGCAG-3' (SEQ ID NO: 6) and 10 pmoles of a biotinylated universal primer complementary to the 5' end of the PCR amplicon 5'-AGCGGATAACAAATTTCACACAGG-3' (SEQ ID NO: 7). Alternatively, the biotinylated universal primer could be 5'-GGCGCACGCCTCCACG-3' (SEQ ID NO: 16). After an initial round of amplification of the target with the specific forward and reverse primer, the 5' biotinylated universal primer was hybridized and acted as a reverse primer thereby introducing a 3' biotin capture moiety into the molecule. The amplification protocol resulted in a 5'-biotinylated double stranded DNA amplicon, which dramatically reduces the cost of high throughput genotyping by eliminating the need to 5' biotin label each forward primer used in a genotyping. Thermal cycling was performed in 0.2 mL tubes or 96 well plate using an MJ Research Thermal Cycler (Waltham, Mass.) (calculated temperature) with the following cycling parameters: 94.degree. C. for 5 min; 45 cycles: 94.degree. C. for 20 sec, 56.degree. C. for 30 sec, 72.degree. C. for 60 sec; 72.degree. C. 3 min.

Detail Description Paragraph:

[0399] PCR primers were synthesized by OPERON (Alameda, Calif.) using

phosphoramidite chemistry. Amplification of the AKAP10 target sequence was carried out in single 50 .mu.l PCR reaction with 100 ng-1 ug of pooled human genomic DNAs in a 50 .mu.l PCR reaction. Individual DNA concentrations within the pooled samples were present in equal concentration with the final concentration ranging from 1-25 ng. Each reaction contained 1.times.PCR buffer (Qiagen, Valencia, Calif.), 200 .mu.M dNTPs, 1U Hotstar Taq polymerase (Qiagen, Valencia, Calif.), 4 mM MgCl<sub>2</sub>, and 25 pmols of the forward primer containing the universal primer sequence and the target specific sequence 5'-AGCGGATAACAATTTCACACA-GGGAGCTAGCTTGGAGATTGC-3' (SEQ ID NO:12), 2 pmoles of the reverse primer 5'-GTCCAATATGCAAACAGTTG-3' (SEQ ID NO: 10) and 10 pmoles of a biotinylated universal primer complementary to the 5' end of the PCR amplicon BIO:5'-AGCGGATAACAATTTCACACAGG-3' (SEQ ID NO: 7). After an initial round of amplification with the target with the specific forward and reverse primer, the 5' biotinylated universal primer can then hybridized and acted as a forward primer thereby introducing a 5' biotin capture moiety into the molecule. The amplification protocol resulted in a 5'-biotinylated double stranded DNA amplicon and dramatically reduces the cost of high throughput genotyping by eliminating the need to 5' biotin label every forward primer used in a genotyping.

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□ 10. Document ID: US 6391558 B1

L6: Entry 10 of 10

File: USPT

May 21, 2002

DOCUMENT-IDENTIFIER: US 6391558 B1

TITLE: Electrochemical detection of nucleic acid sequences

Detailed Description Text (141):

Techniques for allele-specific amplification of Factor V wild-type and mutant genes have been described (Kirschbaum and Foster, 1995). The allele-specific amplification of Factor V genes has been repeated followed with electrochemical detection using Rapid PCR.TM. detection methodology. The forward primer has the same sequence as that reported by Kirschbaum and Foster, except that it is labeled with a biotin at the 5'-end. Two discriminating reverse primers have the same sequences as those reported by Kirschbaum and Foster, but are modified with a 5'-fluorescein. These primers are discriminatory based on homology of the 3'-nucleotide of the reverse primers with either the wild-type or the mutant DNA sequence. PCR amplification was performed as described by Kirschbaum and Foster (1995), and amplification products were captured directly onto sensors through the biotin:avidin interaction. The double-stranded DNA was conjugated with anti-fluorescein HRP, washed and treated with the substrate and mediator to generate a current. PCR amplification products were also monitored by agarose gel electrophoresis to confirm that double-stranded products generated are of the predicted size. Null DNA controls and controls of known genotype were included as well. A high signal is expected only when the discriminating primer finds homology with one of the alleles in the sample.

Detailed Description Text (382):

Techniques for allele-specific amplification of Factor V wild-type and mutant genes have been described (Kirschbaum and Foster, 1995). Therefore, initial experiments were aimed at repeating the allele-specific amplification of Factor V genes, followed with electrochemical detection using our direct detection methodology. The forward primer has the same sequence as that reported by Kirschbaum and Foster, except that it is labeled with a biotin at the 5'-end. Two discriminating reverse

primers have the same sequences as those reported by Kirschbaum and Foster, but are modified with a 5'-fluorescein. These primers are discriminatory based on identity of the 3'-nucleotide with either the wild-type or the mutant Factor V gene sequence. PCR amplification was performed as described by Kirschbaum and Foster, and amplification products were captured directly onto sensors through the biotin:avidin interaction as described. The double-stranded DNA was conjugated with anti-fluorescein HRP, washed and treated with the substrate and mediator to generate a current. PCR amplification products will also be monitored by agarose gel electrophoresis to confirm that double-stranded products generated are of the predicted size. Null DNA controls and controls of known genotype will be included as well. A high signal from normal individuals is expected with only a background signal from homozygous carriers. After conditions for amplification and assay have been determined for Factor V, various types of samples will be tested. Samples from individuals known to be normal for the Factor V gene mutation, heterozygous for the mutation -and homozygous for the mutation (provided by L. Silverman) were analyzed. In a blind study the genotype at the factor V locus was correctly determined in 19 samples.

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